

Extraction of gelatin from fish wastes for use in diets for the goldfish *Carassius auratus*

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Received 29/12/2024

Accepted 16/03/2025

Published 25/06/2025

Abstract

The current study aimed to extract gelatin from the skins of the oriental sole *Brachirus orientalis* using a chemical method. The chemical composition of the extracted gelatin showed a moisture content of 9.66%, a high protein content of 86.11%, a fat content of 1.44%, and an ash content of 2.69%. The results of amino acid analysis of the gelatin protein indicated the presence of 17 amino acids. The highest concentration was for threonine at 72.13 mg/g and glutamic acid at 89.79 mg/g, while lysine and aspartic acid had the lowest concentrations at 18.72 mg/g and 10.44 mg/g, respectively. The results showed a variation in the chemical composition between the different diets. The highest protein content was observed in the T3 diet, with a value of 31.91%, compared to the other treatments C, T1, and T2, which recorded values of 30.25%, 30.74%, and 31.52%, respectively. The highest values for final weight, total weight gain, daily growth, specific growth rate, relative growth, and feed conversion ratio were recorded in the T3 diet, with values of 171.79 g, 84.86 g, 1.13 g/day, 4.02%/day, 1.30%, and 3.21, respectively. The lowest values for these parameters were observed in the control diet C, with values of 131.97 g, 45.09 g, 0.60 g/day, 2.71%/day, 0.69%, and 5.24, respectively. The results demonstrated that the use of gelatin in T1, T2, and T3 diets provided good growth compared to the control diet, which lacked gelatin addition. The T3 diet outperformed all other diets in growth parameters, indicating the success of using protein products in fish diet across all treatments. However, the T3 diet was the best, with a significant difference ($P < 0.05$) compared to other treatments. The T3 diet also showed the best physical properties (density, buoyancy, sinking speed, and disintegration) compared to the other diets. Statistical analysis revealed significant differences between treatments ($P < 0.05$).

Keywords: Fish diet, gelatin, Fish nutrition, goldfish .



Introduction

Fish supplies from fishing and aquaculture operations tend to increase year by year, leading to a rise in the amount of secondary waste produced, which ranges from 13% to 50% of the original raw material. This includes heads, skins, fins, tails, internal organs, as well as fish unsuitable for direct processing, which are often caught unintentionally (See *et al.*, 2010). The accumulation of fish waste and its lack of direct utilization create an environmental and health problem, along with negative effects due to its high organic content (Prasanthi *et al.*, 2016). Therefore, its use is important to increase the commercial value of caught or farmed fish and to reduce and prevent environmental pollution caused by its disposal (Nurilmala *et al.*, 2022). The nutritional value of fish waste is related to its content of proteins, fats, carbohydrates, and good amounts of vitamins and minerals (Tesfaye *et al.*, 2018). Fish waste can be used as a major component in feed, as it represents an important and primary source of high-quality protein due to its essential amino acids necessary for growth (Vineis *et al.*, 2019). Due to its digestibility and high protein content, fish waste is a good source for producing secondary products using chemical, enzymatic, and physical methods, resulting in compounds with good functional properties (Abdulwahab *et al.*, 2023). Gelatin, a gelling agent extracted from the partial hydrolysis of collagen, which is the main protein in skin, connective tissues, bones, and cartilage, can also be produced from certain fish by-products (Al-Nimry *et al.*, 2021). Zaelani *et al.* (2019) noted that fish by-products such as skins, bones, skeletal structures, fins, and skin are the basis for producing fish gelatin, as they contain a significant amount of collagen, reaching up to 30% of the proteins in these by-products. Gelatin is used in various food, pharmaceutical, and engineering applications (Wang *et al.*, 2018). It serves as a gelling agent, film-forming agent, and coagulation agent in many food products and confectioneries (Wu *et al.*, 2023). Additionally, gelatin has medical applications in hydrogels, wound healing, and drug delivery, and it also finds uses in engineering such as in nanofibers, air filters, and biomaterials (Al-Shatty *et al.*, 2016; Alipal *et al.*, 2021). Many food additives are used in fish feeds for various purposes; some improve the functional properties of the feed, such as gelling agents, binders, antioxidants, attractants, or supply essential nutrients like vitamins, minerals, amino acids, cholesterol, and phospholipids (Orisasona, 2018). This, in turn, affects how the feed is formulated to be palatable to fish, improving their intake, growth, and reducing economic costs (Saalah *et al.*, 2010). Wheaton *et al.* (2002) discussed the potential use of gelatin extracted from bones as a protein source and binding agent in many applications. They found that its addition to food improved the food's appearance and nutritional value due to its rich protein and essential amino acids content, making it highly nutritious (Nurilmala *et al.*, 2020). Al-Dubakel *et al.* (2014) mentioned that gelatin extracted from fish waste using an acidic method can be used in fish feeds for fish in intensive and semi-intensive aquaculture systems that rely on artificial feeding, which significantly impacts fish production. For economic reasons, the current study aims to utilize fish waste, as it is an available and inexpensive agricultural

by-product, as a partial replacement for fishmeal in compound feeds for goldfish. This research aims to enhance the sustainability of fish production by recycling agricultural waste into value added materials and to reduce environmental pollution caused by large amounts of fish waste being disposed of on land or into water bodies, which has become a challenging and costly process, increasing environmental pollution and the risk of diseases in humans and animals.

Materials And Methods

Raw Materials for Gelatin Preparation

In the study, fish by-products especially fish skins from the oriental sole *Brachirus orientalis* were used as the primary source for gelatin extraction. These by-products were obtained from local markets in Basrah city as incidental residues of fresh fish cleaning processes. After obtaining the samples, they were thoroughly rinsed with water, subsequently cut into small pieces and mixed well. A representative random sample of the residues was isolated for chemical analysis, while the remaining portion was placed in polyethylene bags and stored at $(-12^{\circ}\text{C} \pm 2)$ until the extraction process.

Extraction of Gelatin from Fish Skin

Gelatin was extracted following the method of Grossman and Bergman (1992). Fish by-products (skins) were cut into small pieces using sharp knives. Subsequently they were thoroughly rinsed with tap water and transferred in 10 litre plastic containers. They by-products were treated with a 0.2% sodium hydroxide solution for 50 minutes at room temperature, then rinsed completely with tap water for 10 minutes, followed by washing with distilled water. A second treatment was carried out using a 0.2% sulfuric acid solution at a ratio of (1:6) (w:v) for 50 minutes at room temperature. After removing the sulfuric acid, samples were immediately soaked in a 1% citric acid solution at a ratio of (1:6) (w:v) for 50 minutes at room temperature. The samples were then washed thoroughly with tap water and subsequently with distilled water for 10 minutes using mesh containers to remove the acidic solutions. After washing, they were mixed in an electric blender for one minute to homogenize thereafter combined with distilled water at a ratio of (1:2.5) (w:v) and heated to 45°C for 18 hours to facilitated gelatin exrtaction. The samples were filtered using a cloth to remove fats and contamination. The filtrate was concentrated using a rotary evaporator at 70°C for 2 hours. The resulting product was dried in a conventional oven at 50°C , grounded using an electric grinder and stored in the refrigerator at $4-8^{\circ}\text{C}$ for testing and further use.

Chemical composition of (Fish skin, Gelatin, and prepared Feeds)

Moisture contents were assessed by oven drying at 105°C , The ash percentage was determined by combustion the samples in a muffle furnace at 525°C for 16 hours, Protein content was determined using the semi-micro Kjeldahl method, with the result multiplied by a conversion factor of 6.25 to, and Lipid content was determined using

Soxhlet extraction according to the method described in the study of Egan *et al.* (1988), The carbohydrate percentage was calculated mathematically according to AOAC (2000).

Estimation of Amino Acids

Amino acid profiles of prepared gelatin was determined according to Vidotti *et al.* (2003) An ion exchange column and post-column ninhydrin derivatization were used for analysis, utilizing the Visible-UV Detector -6 Av uv -Spd Shimadzu in an automatic analysis system. High-performance Liquid Chromatography (HPLC) equipment, under the supervision of the Ministry of Science and Technology in Baghdad, Iraq, was employed for this purpose.

Feed Formulation

After determining the quantities of the basic feed materials utilised for the preparation of the fish feeds under examination, as indicated in (Table 1), the feed ingredients were carefully crushed and screened through a sieve with 2 mm apertures. The materials were subsequently mixed thoroughly according to the stated ratios to ensure homogeneity. Four experimental feeds were prepared: T1 containing 1% gelatin added, T2 containing 1.5% gelatin added, T3 containing 2% gelatin added, and a control sample (C) free of gelatin. Approximately 100 ml of boiling water was added to every 250 grams of the mixture. After mixing thoroughly, the temperature of the mixture was raised to 80°C and then allowed to cool. Vitamins and minerals were added after cooling. The feed paste was then shaped into pellets using a Braun meat grinder with 4 mm diameter openings. The extruded feed was left to air-dry in the laboratory for 48 hours with continuous stirring to remove moisture and ensure complete drying. The prepared feed was then stored in 1 kg plastic containers and kept in the refrigerator until use.

Table 1. Formulation Ingredients (%) of experimental diets.

Ingredient	Treatment			
	C	T1	T2	T3
Fish meal	20	20	20	20
Soybean meal	20	20	20	20
Barley flour	20	19	18.5	18
Yellow corn meal	20	20	20	20
Wheat bran	15	15	15	15
Vegetable oil	3	3	3	3
premix	2	2	2	2
Gelatin	0	1	1.5	2
	100	100	100	100

Fish and Experimental System

Goldfish (*Carassius auratus*) were purchased from a local ornamental fish supplier in Basrah city during November 2023, with an average weight of $10.85 \text{ g} \pm 20.0$. The experimental fish rearing system was designed using a closed recirculating system in the Fish Farming Laboratory, Department of Fisheries and Marine Resources, College of Agriculture at the University of Basrah. At the beginning of the experiment, the fish were distributed with eight fish per tank. Fish were acclimated to the experimental conditions for ten days, during which they were fed a standard diet.

Feeding experiment

Fish growth

The feeding experiment lasted for 70 days, starting from December 10, 2023, until February 18, 2024. During this period, the fish were fed at a rate of 5% of their body weight, with two meals per day (each meal amounting to 2.5% of their body weight) at 8-9 AM and 1-2 PM. The fish were weighed every 14 days to adjust the amount of feed, and 30% of the water was changed every 14 days. Fish growth parameters, namely weight gains (TWG) and daily weight gains (DWG), were calculated following the method described by Sevier *et al.* (2000) as follows:

$\text{TWG (g/fish)} = \text{Final weight} - \text{Initial weight}$

$\text{DWG (g/fish/day)} = \text{TWG} / \text{time (day)}$

Relative (RGR) and specific (SGR) growth rates were calculated as described by

$\text{RGR (\%)} = \text{TWG} / \text{Initial wt.} \times 100$

$\text{SGR (\%/day)} = (\ln \text{ final wt.} - \ln \text{ Initial wt.}) / \text{time (day)} \times 100$

Feed conversion ratio (FCR), protein intake (PI) and protein efficiency ratio (PER) were calculated using the method applied by Tacon (1990) as follows:

$\text{FCR} = \text{Consumed feed (g)} / \text{TWG (g)}$

$\text{PI (g/fish)} = \text{Consumed feed (g)} \times \text{Feed protein content (\%)}$

$\text{PER (\%)} = \text{TWG} / \text{PI}$

Statistical analysis

The growth experiment was designed according to the complete randomized design (CRD) with four treatments, each with three replications. The same statistical analysis approach was applied to other studied feeding and growth parameters. The significant differences between treatment means were determined using the least significant difference (LSD) test. All statistical analyses were conducted using the Statistical Package for Social Sciences (IBM SPSS) version 26.0.

Results

Table (2) shows the examined fish waste and the chemical composition of the produced gelatin. The moisture content in the waste was 70.88%, while the protein content was 19.42%. The fat and ash contents were 4.86% and 4.58%, respectively. In

comparison, the prepared gelatin had a moisture content of 9.66%, a high protein content of 86.11%, and fat and ash contents of 1.44% and 2.69%, respectively.

Table 2. Proximate composition (%) of fish waste and gelatin prepared.

	Moisture	Crude protein	Crude lipid	Ash
Fish waste	70.88	19.42	4.86	4.58
Gelatin	9.66	86.11	1.44	2.69

The results in Table (3) present the amino acid analysis using HPLC for the gelatin prepared from fish waste. The results showed that the extracted gelatin contained 17 amino acids, with varying concentrations. Among the essential amino acids, there were 9 types, with the highest value being for threonine at 72.13 mg/gram, while lysine had the lowest concentration at 18.72 mg/gram. Conversely, the non-essential amino acids included 8 types, with glutamic acid having the highest concentration at 89.79 mg/gram, and aspartic acid having the lowest at 10.44 mg/gram. It was observed that tryptophan was absent in the prepared gelatin, which is a distinguishing feature indicating its purity and the absence of other proteins. The remaining amino acids varied in their concentrations in the gelatin.

Table 3. Amino acid profiles of gelatin prepared.

Amino Acid			
Essential Amino Acids (EAA)	Histidine	His	47.35
	Arginine	Arg	64.94
	Threonine	Thr	72.13
	Valine	Val	44.99
	Methionine	Met	53.93
	Isoleucine	Iso	33.29
	Leucine	Leu	33.47
	Phenylalanine	Phe	31.31
	Lysine	Lys	18.72
Σ EAA			400.16
Non-Essential Amino Acids (NEAA)	Aspartic acid	Asp	10.44
	Glutamic acid	Glu	89.79
	Serine	Ser	67.94
	Glycine	Gly	49.58
	Alanine	Ala	77.85
	Proline	Pro	33.44
	Tyrosine	Tyr	49.24
	Cysteine	Cys	36.96
Σ NEAA			415.26

EAA, Essential Amino Acids; NEAA, Non-Essential Amino Acids.

Table (4) shows the chemical composition of the studied diets, with results indicating variation in chemical composition among the different diets. The highest moisture content was 5.73% in Diet C, while it decreased to the lowest value of 5.47% in Diet T3, compared to the moisture percentages in Diets T1 and T2, which were 5.67% and 5.58%, respectively. Regarding protein content, the highest value was 31.91% in Diet T3, differing from the other treatments C, T1, and T2, which had values of 30.25%, 30.74%, and 31.52%, respectively. For fat content, the results showed that Diet T1 had the highest value at 4.73%, differing from the other treatments C, T2, and T3, which had fat percentages of 4.46%, 4.59%, and 4.66%, respectively. All treatments exhibited varying levels of ash content depending on the type of diet, with values ranging from 6.45% to 6.42%, 6.13%, and 6.27% for diets C, T1, T2, and T3, respectively. Carbohydrate values showed that Diet T3 had the lowest percentage at 51.69%, differing from the other treatments, with carbohydrate values of 53.08%, 52.44%, and 52.18% for diets C, T1, and T2, respectively.

Table 4. Proximate composition of fish diets.

Treatment	Moisture	Ash	Crude lipid	Crude protein	Carbohydrate
C	5.73	6.45	4.46	30.25	53.08
T1	5.67	6.42	4.73	30.74	52.44
T2	5.58	6.13	4.59	31.52	52.18
T3	5.47	6.27	4.66	31.91	51.69

Table (5) shows the initial weight (grams), final weight (grams), weight gain rate (grams), total weight gain (grams), specific growth rate(%/day), relative growth rate (%), feed conversion ratio, and protein efficiency ratio (%) for the experimental fish. The results indicated that the highest values for final weight, total weight gain, daily growth, specific growth rate, and relative growth rate were observed in Diet T3, with values of 171.79 grams, 84.86 grams, 1.13 grams/day, 4.02%/day, and 1.30%, respectively. The lowest values were recorded in the control diet C, with values of 131.97 grams, 45.09 grams, 0.60 grams/day, 2.71%/day, and 0.69%, respectively. The results demonstrated that the use of gelatin in Diets T1, T2, and T3 resulted in better growth compared to the control diet without additives. Diet T3 outperformed all other diets in growth parameters, indicating the success of using protein products in fish nutrition across all treatments, with Diet T3 being the most effective with a significant difference ($P<0.05$) compared to the other treatments. The best feed conversion ratio was observed in Diet T3, with a value of 3.21 and a significant difference ($P<0.05$) compared to the other treatments, which had feed conversion ratios of 5.24, 4.32, and 3.61 for Diets C, T1, and T2, respectively. Statistical analysis showed significant differences ($P<0.05$) in feed conversion ratio among the treatments. The highest protein efficiency ratio was found in Diet T3 at 1.01, while the lowest was 0.63 in the control diet C. The protein efficiency

ratios for Diets T1 and T2 were 0.76 and 0.89, respectively, with significant differences ($P < 0.05$) among the treatments.

Table 5. Feeding and growth parameters of experimental fish.

Parameter	Treatment			
	C	T1	T2	T3
Initial weight (g)	86.87a	86.19a	87.32a	86.92a
Final weight (g)	131.97a	143.67a	159.24bc	171.79c
Weight gain (g)	45.09a±6.728	57.48b±8.915	71.92c±3.186	84.86d±0.558
RGR (%)	0.69a±0.093	0.89b±0.160	1.09c±0.052	1.30d±0.025
DGR	0.60a±0.090	0.76b±0.119	0.95c±0.042	1.13d±0.007
SGR (%/day)	2.71a±0.456	3.31b±0.373	3.75bc±0.077	4.02c±0.015
FCR	5.24a±0.690	4.32b±0.619	3.61bc±0.125	3.21c±0.055
FCE	19.26a±2.358	23.41b±3.161	27.66bc±0.946	31.13c±0.531
PER	0.63a±0.079	0.76ab±169.395	0.89bc±0.031	1.01c±0.015
PI	7066.41a±220.732	7480.23ab±0.103	7990.82bc±82.631	8401.02c±94.593

*Different letters within one column indicate the presence of significant differences at the level ($P < 0.05$).

Table (6) presents the physical properties of the studied diets. The diet without the binder, C, recorded the highest density at 1.4 g/cm³, while the densities of the diets containing gelatin were 1.2, 1.1, and 0.9 g/cm³ for Diets T1, T2, and T3, respectively. Statistical analysis showed significant differences among the treatments ($P < 0.05$). Diet T3 exhibited the longest floating time at 14.92 seconds, while Diet C had a floating time of only 7.5 seconds, significantly different from the other diets. The floating times for Diets T1 and T2 were 8.11 and 12.69 seconds, respectively. This was reflected in the sinking speed, with Diet T3 showing the highest sinking speed at 2.63 seconds/cm, significantly different from the other treatments ($P < 0.05$). The sinking speeds for Diets C, T1, and T2 were 1.68, 1.75, and 1.92 seconds/cm, respectively. The results indicated that the addition of gelatin significantly reduced the water absorption of the feed pellets during immersion. Water absorption values increased for all treatments, with Diet C recording the highest water absorption at 1.30%, significantly different from the other treatments ($P < 0.05$). The other diets showed similar patterns, with values of 1.28, 1.22, and 1.15 for Diets T1, T2, and T3, respectively. The pellet disintegration rates for the gelatin-based diets, T2 and T3, were 0%, while Diets C and T1 had disintegration rates of 1.05% and 0.66%, respectively, with significant differences from the other treatments ($P < 0.05$).

Table 6. Physical properties of fish diets.

Treatment	Parameter			
	Density g/cm ³	Floating time/sec	Sinking Speed %	Disintegration rate %
C	1.4c±0.00	7.5a±0.429	1.3c±0.053	1.05c±0.037
T1	1.2bc±0.00	8.11a±0.196	1.28bc±0.078	0.66b±0.577
T2	1.1b±0.00	12.69b±0.936	1.22ab±0.044	0a±0.00
T3	0.9a±0.00	14.92c±0.055	1.15a±0.029	0a±0.00

Discussion

The chemical composition results of fish waste were consistent with the study of Muyonga *et al.* (2004) on the chemical composition of Nile perch fish skins, where the protein content was 21.6% and 20.3%, moisture content was 68.4% and 72.7%, fat was 6.8% and 4.96%, and ash was 6% and 3.7% for adult and juvenile fish, respectively. The differences in the chemical composition of the waste were in line with Jakhar *et al.* (2012), who studied the chemical composition of Blackspotted Croaker (*Protonibea diacanthus*) waste, finding moisture at 75.80%, ash at 1.06%, fat at 2.48%, and protein at 20.63%. These results also matched the findings of Viles *et al.* (2013), who reported the chemical composition of Rainbow trout (*Oncorhynchus mykiss*) waste, where moisture ranged from 49.73% to 54.72%, fat ranged from 41.74% to 30.22%, protein ranged from 17.15% to 7.92%, and ash was between 1.28% and 0.8%. Jaber and Najim (2022) observed that the moisture content of common carp (*Cyprinus carpio* L.) waste was 71.39%, protein was 17.91%, fat was 6.76%, and ash was 3.37%. Furthermore, other studies reported moisture, protein, fat, and ash content in fish waste ranging from 63.69 - 68.74%, 12.03 - 18.30%, 7.11 - 22.98%, and 1.03 - 5.85%, respectively (Al-Hilphy *et al.*, 2020; Salih *et al.*, 2021). Renuka *et al.* (2017) noted that fish waste is a good source of protein at 58% dry matter, fat at 19%, and minerals, and possesses high digestibility and a high content of amino acids and fatty acids. Therefore, it is a good source for producing protein concentrates and stabilizers like gelatin, which are used as protein sources in fish feed and various applications (Al-Noor *et al.*, 2013; Najim *et al.*, 2015).

The variation in the chemical composition of gelatin produced aligns with several studies. For instance, a study by Khiari *et al.* (2011) on the chemical composition of gelatin extracted from mackerel fish waste using different organic acids found that the highest protein content was 89.4%, with fat content at 0.8%, moisture at 7.7%, and ash at 1.6%. In another study by Atma (2017), which compared the chemical composition of various types of gelatin prepared from different warm-water fish bones, it was found that gelatin from Pangasius catfish bones had the highest protein content at 87.3%. Moisture content ranged from 4.1 - 11.43%, ash content from 2.6 - 11.17%, and fat content from 0.01- 5.16%. Alfaro *et al.* (2014) indicated that the recommended maximum ash content for gelatin is 2.6%, though gelatin derived from bones may have higher ash content. These results are consistent with a study by Wu *et al.* (2023) on the physical properties

and chemical composition of gelatin prepared from different fish sources, which showed protein content ranging from 90.9 - 99.9% depending on the raw material. Nurilmala *et al.* (2024) observed that gelatin prepared from tilapia scales contained 10.26% moisture and 0.16% ash. In a study by Lestari *et al.* (2024) on gelatin extracted from catfish skins, they found protein content ranging from 88.92 - 93.22% and ash content from 0.68 - 0.37%. According to Bordignon *et al.* (2012), gelatin contains moisture content ranging from 9 - 14%. Studies by Bigi *et al.* (2004), Jakhar *et al.* (2012), and Wahyuningtyas *et al.* (2019) on the physical and chemical properties of gelatin from Red Snapper scales showed considerable variation in moisture, protein, and ash content, with minimal differences in fat content. Protein content ranged from 78.79- 87.27%, moisture from 4.5 - 2.08%, ash from 0.180 - 0.37%, and fat from 0.0 - 0.002%.

Regarding the amino acid content, it was observed that tryptophan was absent in the prepared gelatin. This is a distinctive feature indicating its purity and the absence of other proteins (Boran and Regenstein, 2010). The remaining amino acids varied in their concentrations in the gelatin, and these results are consistent with a study by Duan *et al.* (2011) which reported variations in the amount and proportion of amino acids in gelatin extracted from carp skins depending on the season, finding that glycine made up approximately 33% of the total amino acids. Current results also align with Badii and Howell (2006) who noted clear variations in the amino acid composition and proportions among gelatin types derived from different fish sources. The variation in amino acid proportions depending on the type of gelatin is supported by several researchers. Khiari *et al.* (2011) indicated that the amino acid composition and proportions of gelatin derived from fish waste varied across different types of gelatin, with glycine being the predominant amino acid, along with high contents of proline, hydroxyproline, alanine, and glutamic acid. According to Go´mez-Guille´n *et al.* (2022) the amino acid content in gelatin extracted from different fish species varies significantly, with the amino acid composition primarily consisting of 33% glycine, 20% proline and hydroxyproline, and 11% alanine. Alfaro *et al.* (2014) found results consistent with Kim and Park (2004) who observed slight differences in amino acid content depending on the type of organic acid used, noting particularly reduced levels of proline and hydroxyproline in gelatin extracted with lactic acid. These findings are consistent with Binsi *et al.* (2009) who studied the amino acid composition of Bigeye snapper skins and found a notable variation in proportions, with glycine being higher than other amino acids. The current results also align with several studies showing clear differences in amino acid proportions and quantities in gelatin, including Cho *et al.* (2006) Arnesen and Gildberg (2002) and Jellouli *et al.* (2011). The results are also similar to those of Izzati *et al.* (2017) who studied the amino acid content in collagen derived from Nile tilapia skins and Zeng *et al.* (2015) who investigated hydrophobic amino acids in collagen from tilapia scales. Some studies have indicated that the presence of proline and hydroxyproline is crucial for the structural integrity of collagen and plays a role in stabilizing the triple helix through hydrogen bonds in the hydroxyl

groups of hydroxyproline (Huang *et al.*, 2011). Overall, glycine, proline, hydroxyproline, and alanine are the predominant amino acids, with respective percentages of 33, 20 and 11 (Mahmood *et al.*, 2016).

The chemical composition of diets and feed additives is one of the most important factors that can influence the chemical composition of fish bodies (Al-Janabi *et al.*, 2022). Aydin and Gumus (2013) indicated that the moisture content in the diets ranged from 7.61 to 8.63%, while the protein content was 30.17%. These results are consistent with the study by Taher *et al.* (2022), where the protein content in the diets prepared for feeding grass carp (*Ctenopharyngodon idyll*) was 30.11%. Ayuba and Iorkohol (2010) observed significant variation in the chemical composition of different studied diets, which was confirmed by Al-Tameemi (2015) in his assessment of five different types of fish feeds, with values similar to those reported by Amtul and Amna (2012). The variation in the chemical composition of the diets aligns with the findings of Jassim *et al.* (2024) who found differences in protein, fat, and other components of the diet depending on the preparation method. Abdulwahab *et al.* (2023) also reported variations in the chemical composition of different types of manufactured fish feeds, noting that the differences were due to the type of raw materials used in the manufacturing process.

The weight, total weight gain, and growth rate criteria are among the essential and practical standards widely used to evaluate the quality of different feeds and feed additives. These criteria represent the outcome relied upon to achieve the best results from high-quality feed sources (Hepher, 1988). The results indicated that all treatments containing gelatin significantly outperformed the control treatment. This is likely because gelatin derived from animal collagen, regardless of its source, contains a high percentage of amino acids ranging from 25% - 50%, making it highly nutritious (Alfaro *et al.*, 2014). Gelatin contains 98% protein and high levels of amino acids such as glycine (26–34%), proline (10–18%), hydroxyproline (7–15%), alanine (8–11%), arginine (8–9%), glutamic acid (10–12%), and aspartic acid (6–7%) (Mahmood *et al.*, 2016). Wu *et al.* (2023) noted that gelatin is used to enhance nutritional value due to its high protein content, improving the elasticity, firmness, and stability of food products, in addition to significantly enhancing functional properties. It was found that adding gelatin to diets significantly affected the chemical composition, particularly increasing protein content compared to the control diet. Gelatin acts as a binding agent in the diet and as a pure protein source, thereby improving the feed conversion ratio by using the binding agent (Wheaton *et al.*, 2002). The findings of the current study are consistent with those of Al-Dubakel *et al.* (2014), who investigated the use of gelatin extracted from fish waste in the formulation of diets for common carp. They found that all treatments containing different levels of gelatin outperformed the control diet without gelatin, as gelatin is a rich source of protein, essential amino acids, vitamins, and minerals. Similarly, Quintana *et al.* (2008) studied the effect of using two types of moist artificial feeds with gelatin on the dietary performance and growth of common octopus, showing that this dietary

system improved growth performance and feed conversion, positively affecting other growth criteria. Tong *et al.* (2023) studied the possibility of replacing fishmeal with collagen prepared from fish waste in the diets of Triploid crucian carp at varying levels to evaluate the effects on muscle quality and fish glycolipid metabolism. Results indicated that increasing the level of collagen replacement above 4% led to a reduction in the specific growth rate (SGR) and a negative impact on growth but improved muscle quality and reduced glycolipid levels. As observed in the current study, the artificial diets used by Cerezo Valverde *et al.* (2008) primarily consisted of natural food clumped with a binding agent, which positively influenced the nutritional value, palatability of the feed, weight gain, growth rate, and feed conversion efficiency. Gao *et al.* (2019) noted that using gelatin at varying levels as a dietary supplement in gibel carp (*Carassius gibelio*) diets improved feed quality, increased amino acid levels in the feed, and enhanced growth. Arguello-Guevara and Molina-Povedo (2012) found that the use of binding agents in the manufacture of *Litopenaeus vannamei* shrimp diets led to better growth and improved feed conversion efficiency.

The current study demonstrated that the use of binding agents in feed improved the physical and chemical properties of the diets, in addition to their role in increasing feed intake and growth indicators. The use of binding agents at levels lower than 2% is the recommended percentage in most fish diets (Lovell, 1989). The results showed that diets containing gelatin as a source outperformed the control diet in physical characteristics, such as pellet density, floating time, and sinking speed, which is important for the stability of diets in water, as most fish feed near the surface and can thus benefit from these characteristics (Jobling, 1993). The current results are consistent with Ward and Courts (1977), who indicated that gelatin is a protein capable of forming a gel between 30°C and 35°C, dissolving upon heating in solutions, solidifying upon cooling, and becoming semi-solid and highly viscous in water, appearing as a floating solution. The results also showed that water absorption was lower in gelatin-containing diets compared to the control diet. This is attributed to the greater cohesion of the pellets and fewer pores, which limit water penetration between components, thus reducing water absorption and increasing resistance to disintegration (Behnke, 2001). McWilliams (2001) stated that gelatin is an organic binding agent used at 1.5% to 4% in aquatic feeds, easily forming hydrogen bonds with water due to the presence of many accessible polar regions. It swells upon water absorption and, when it becomes a gel, compresses and arranges into continuous molecules, becoming more organized and orderly (Hudson, 1994). Misra (2002) mentioned that the water absorption of pellets over a certain period can be considered an indicator of the quality of those pellets regarding their resistance to disintegration in water. He noted that the quality of feed pellets can be evaluated by testing the water absorption rate of the pellets and their stability in water. The current study found that the use of gelatin as a binding agent positively affected growth indicators such as weight gain, relative and specific growth rates, absolute gain, and feed intake, as high values were recorded in diets containing the binding agent. This

improvement is attributed to the enhanced characteristics of the diet and increased palatability of fish (Fagbenro and Jaunted, 1995). Palma *et al.* (2008) used gelatin and carboxymethyl cellulose in fish feed production, noting that binding agents increased pellet durability by reducing voids and increasing steam gelatinization, which in turn reduced feed solubility and enhanced the physical properties of the feed (Zhu *et al.*, 2010). Gao *et al.* (2019) observed that using varying levels of gelatin as a dietary supplement in the diets of gibel carp (*Carassius gibelio*) improved feed quality, reduced solubility, and increased pellet cohesion strength. Arguello-Guevara and Molina-Povedo (2012) studied the effects of binding agents in the manufacture of *Litopenaeus vannamei* shrimp feeds, which improved stability properties in water. Lal *et al.* (2023) discovered that the use of binding agents in fish diets led to increased gel strength due to the formation of hydrogen bonds and increased binding of protein tertiary structures, thereby enhancing water stability. Karim *et al.* (2024) also revealed that binding agents performed better in maintaining the physical properties of diets, such as density, sinking speed, and disintegration rate, ultimately reducing economic losses in fish feeds.

In conclusion, it appeared that gelatin could act as a suitable protein ingredient in fish diets supporting fish feeding and growth parameters. In addition, the use of binding agents, especially protein-based ones like gelatin, is essential for maintaining feed quality and minimizing nutrient loss, as well as improving the durability of feed pellets and reducing disintegration rates. This, in turn, helps limit water pollution and economic losses in aquaculture.

Conclusion

The conclusion should be included at the end of the manuscript. It is important to thank individuals or institutions that have provided support or assistance in any way during the completion of the study or research.

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استخلاص الجيلاتين من مخلفات الأسماك لاستخدامه في النظام الغذائي للسكة الذهبية

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تاريخ الاستلام: 2024/12/29 تاريخ القبول: 2025/03/16 تاريخ النشر: 2025/06/25

المستخلص

هدفت الدراسة الحالية الى إنتاج الجيلاتين من جلود أسماك المزلك *Brachirus orientalis* بالطريقة الكيميائية، أن التركيب الكيميائي للجيلاتين الخام فقد بلغت نسبة الرطوبة 9.66 % وأحتوى نسبة عالية من البروتين 86.11 % أما نسبة الدهن فقد بلغت 1.44 % والرماد 2.69 %، كما أشارت النتائج عند تحليل محتوى الأحماض الأمينية لبروتين الجيلاتين أحتواء الجيلاتين المستخلص على 17 حامضاً أمينياً وقد تباينت الأحماض الأمينية بنسب تواجدتها، أن أعلى تركيز كان للأحماض الأمينية الثريونين 72.13 ملي غرام/غرام و حامض الكلوتامك 89.79 ملي غرام/غرام فيما كانت نسبة الحامض اللايسين والحامض الأميني الأسبارتك منخفضة وبمقدار 18.72 و 10.44 ملي غرام/غرام على التوالي، بينت النتائج وجود تباين في التركيب الكيميائي بين أنواع العلائق إذ بلغت أعلى قيمة للبروتين 31.91 % في عليقة T3 وبفارق عن بقية المعاملات C و T1 و T2 والبالغة 30.25 و 30.74 و 31.52 % على التوالي، سجلت النتائج أعلى قيمة للوزن النهائي والزيادة الوزنية الكلية والنمو اليومي والنمو النوعي والنمو النسبي ومعدل التحويل الغذائي أعلى قيمها في عليقة T3 حيث بلغت 171.79 غم، 84.86 غم، 1.13 غم/يوم، 4.02 %/يوم، 1.30 %، 3.21 على التوالي، وكانت أقل قيم لها في عليقة السيطرة C 131.97 غم، 45.09 غم، 0.60 غم/يوم، 2.71 %/يوم، 0.69 %، 5.24 وبترتيب نفسها. أثبتت النتائج أن أستعمال الجيلاتين في عليقة T1، T2، T3 أعطت نمواً جيداً بالمقارنة مع عليقة السيطرة الخالية من الإضافات وتبين أن عليقة T3 قد تفوقت على جميع العلائق الأخرى في معايير النمو وهذا يدل على نجاح عملية أستعمال المنتجات البروتينية في تغذية الأسماك ولجميع المعاملات ولكن عليقة T3 كانت هي الأفضل وبفارق معنوي ($P < 0.05$) عن بقية المعاملات. كما سجلت عليقة T3 أفضل صفات فيزيائية (الكثافة، الطفو، سرعة الغطس والتفتت) مقارنة ببقية العلائق وبينت نتائج التحليل الإحصائي فروقاً معنوية بين المعاملات ($P < 0.05$).

الكلمات المفتاحية: علائق اسماك، الجيلاتين، تغذية الأسماك، السمكة الذهبية.